In every one of the 10 experiments, the logarithm of the concentration of radioactive phosphorus left in the blood was inversely proportional to time, the slope of the plot relating the two from zero time to 1 hour varying from −0.91 to −3.06. At the beginning of the experiment the average number of counts per milligram of dried blood was 83, and at the end of 1 hour 97.6% of the radioactive phosphorus had disappeared from the blood. A few experiments with the lobster, *Homarus americanus,* gave essentially the same results except that the rate of disappearance was much slower.

These preliminary tests indicate that radioactive phosphorus will be useful in studying various problems in the physiology and biochemistry of the blood and tissues of *Limulus* and other invertebrates, just as it has been in the vertebrates. Several such problems are now being studied.

13254

Liberation of Heparin by Trypsin.

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As pointed out in a preceding report[1] the intravenous injection of trypsin into various animals produces effects remarkably similar to those of anaphylactic shock in the corresponding animals. It was first emphasized by Biedl and Kraus[2] that defective clotting of the blood is a common and conspicuous feature of anaphylaxis in dogs and it has been shown by numerous workers since that delayed coagulation to complete incoagulability of the blood occurs in other laboratory animals during anaphylaxis, although it may not occur so regularly. We have observed the clotting time of the blood in various animals after the intravenous injection of trypsin. We have observed definitely delayed coagulation to complete incoagulability in 8 of 10 dogs after 1 to 2 mg per kilo (Table I) and in 3 of 4 rabbits after 3 to 7 mg per kilo (Table II). We have also observed

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1 Dragstedt, C. A., and Rocha e Silva, M., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 420.

2 Biedl, A., and Kraus, R., *Wien. Klin. Wchnschr.*, 1909, **22**, 363.

markedly delayed coagulation in cats, rats and guinea pigs, but the observations do not permit a definite statement as to the frequency of its occurrence in these animals. The development and duration of the coagulation defect were carefully followed in dogs. After the intravenous injection of 1 to 2 mg per kilo, the clotting defect may appear within 2 minutes, is usually well in evidence by 4 minutes, may continue to increase in degree until 10 to 15 minutes and then usually declines gradually so that normal coagulability may be restored anywhere from 20 to 60 minutes after the injection if the animal survives. That this loss of coagulability is not due to a direct effect of trypsin upon the blood is indicated by the fact that corresponding amounts of trypsin added to blood *in vitro* has no appreciable effect upon its clotting ability.

TABLE I.
Effect of Intravenous Injection of Trypsin on Clotting Time of Blood in Dogs.

Exp.	Trypsin mg/kilo	Clotting time (min) prior to injection	Clotting time of blood at intervals after injection of trypsin 2′	4′	6′	10′	15′	20′	30′	Reaction
I	2*	3		3		3				Mild
II	3*	3	9					3.5		Severe
III	10*	2.5	5							Moderate
IV	5*	2	4							Mild
VII (1)	1.9	3	5							Severe
(2)	2.0	5				30				Died
IX (1)	0.8	2	2				3.5			Moderate
(2)	1.0	3.5	>12	>60						Severe
X	1.0	3		3		>10		5		Moderate
XI (1)	1.2	2		>25		>45		20	4	Severe
(2)	1.4	4	6			>30		>25		”
(3)	1.2	>25		∞		>45				”
XII (1)	2.6	2	3	>10	>20	4				”
(2)	2.6	4	>10	>25						”
XIV (1)	1.0	3	>12		15				4	Moderate
(2)	1.5	4	∞		∞				12	Severe
XV	2.0	3.5		3.5		3.5	3.0			Moderate

*Commercial trypsin. All others—crystalline trypsin (Plaut Lab. Preparation).
∞ Blood incoagulable for more than 60 minutes.

TABLE II.
Effect of Intravenous Injection of Trypsin on Clotting Time of Blood in Rabbits.

Exp.	Normal clotting time, min	Trypsin* mg/kilo	Interval, min	Clotting time, min	Remarks
1	4	4	5	4	Dead in 5 min.
2	4	6	10	>15	Severe shock
3	4	7	3	>24 hr	Dead in 3 min.
4	5	5	7	>60	” ” 7 ”
5	4	12.5	Intravascular clotting		” ” 2 ”
6	4	25.0	”	”	” ” 2 ”
7†	4	25.0	10	>90	Moderate shock

*Crystalline trypsin (Plaut).
†10 mg heparin injected prior to trypsin.

The nature of the coagulation defect in anaphylaxis has been studied by numerous workers and a great variety of theories have been postulated as to its mechanism. Beginning with the demonstration by Eagle, Johnston and Ravdin[3] of antithrombic activity in the blood of anaphylactized rabbits and dogs, it now appears to be conclusively proved by the work of Waters, Markowitz and Jaques[4] and Jaques and Waters[5] that abnormal amounts of heparin appear in the blood and are responsible for the changes in clotting properties. Their evidence indicates that the heparin is liberated into the blood from the liver. Jaques and Waters made use of the observations of Chargaff and Olson[6] that protamine combines quantitatively with heparin, and found that the coagulability of the blood of shocked animals could be restored to normal by the addition of suitable amounts of protamine. By titrating various samples of blood with varying quantities of protamine, it was possible to give a quantitative expression to the amount of heparin in the blood. We have found that protamine will restore normal coagulability to blood drawn from either rabbits or dogs after trypsin injections and have made quantitative titrations in some of the dog experiments. The observed coagulability of blood samples drawn at various time intervals after the injection of trypsin was roughly parallel to the protamine equivalents of these samples. In 2 experiments peak values of 0.08 and 0.1 mg of protamine per 0.5 cc of blood, respectively, were obtained. These quantities would indicate that a considerable amount of heparin was present as Jaques, Charles and Best[7] found that 1 mg of protamine neutralizes 0.33 mg of heparin.

If the dose of trypsin is increased significantly beyond that which is necessary to produce a severe to fatal reaction, it may produce an extensive intravascular coagulation. As Eagle and Harris[8] have shown, this is due to a direct effect of trypsin upon the blood and can be demonstrated *in vitro* as well as *in vivo*. Intravenous trypsin, therefore, has a dual effect upon the blood, tending to produce an incoagulability indirectly (by the liberation of heparin) and an increased coagulability directly. The mutual antagonism between these effects can be shown in the following ways: The intravascular

[3] Eagle, H., Johnston, C. G., and Ravdin, I. S., *Bull. Johns Hopkins Hosp.*, 1937, **60**, 428.

[4] Waters, E. T., Markowitz, J., and Jaques, L. B., *Science*, 1938, **87**, 582.

[5] Jaques, L. B., and Waters, E. T., *Am. J. Physiol.*, 1940, **129**, 389.

[6] Chargaff, E., and Olson, K. B., *J. Biol. Chem.*, 1937, **122**, 153.

[7] Jaques, L. B., Charles, A. F., and Best, C. H., *Acta Med. Scand.*, Suppl., 1938, **90**, 190.

[8] Eagle, H., and Harris, T. N., *J. Gen. Physiol.*, 1937, **20**, 543.

coagulation which can be produced in a rabbit by the injection of 12 to 25 mg per kilo of trypsin can be prevented by the prior injection of a large dose of heparin (Table II). Likewise, blood which is incoagulable as the result of an injection of 5 mg per kilo of trypsin can be made to coagulate by the addition of further amounts of trypsin. This latter result demonstrates that the loss of coagulability is not due to a destructive action of trypsin upon any of the blood elements essential to its coagulation and indicates that the intravenous injection of shocking doses of trypsin leads to the liberation of sufficient heparin to more than counterbalance the direct coagulant effect of the injected trypsin.

Summary. The intravenous injection of trypsin in doses of 1 to 2 mg per kilo in dogs, or 3 to 7 mg per kilo in rabbits produces a varying degree of incoagulability of the blood. As the addition of similar concentrations of trypsin to blood *in vitro* does not produce this incoagulability, the latter appears to be an indirect effect. The addition of suitable amounts of protamine restores the coagulability to normal, indicating that the coagulation defect is due to heparin, presumably liberated *in vivo* from various tissues such as the liver.

13255

Effect of Radio-Phosphorus on Blood of Monkeys.*

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Radioactive phosphorus can be administered to animals and man in the form of sodium phosphate in doses sufficient to produce a decrease in the number of white blood cells.[1, 2] In order to obtain further data on the effect of radiophosphorus on the blood of normal animals with respect to the maximum dosage which can be tolerated,

* This investigation was supported by a grant from the John and Mary R. Markle Foundation.

† We wish to thank the various members of the staff of the Radiation Laboratory, whose generous coöperation makes radio-phosphorus available for biological and medical investigations.

1 Scott, K. G., and Cook, S. F., *Proc. Nat. Acad. Sci.*, 1937, **23**, 528.

2 Lawrence, John H., Scott, K. G., and Tuttle, L. W., *The New International Clinics*, 1939, 35-58.